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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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TITLE OF THE INVENTION (500 characters max)

NOVEL ANTI-DC-SIGN ANTIBODIES

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Respectfully submitted,

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CERTIFICATION UNDER 37 C.F.R. § 1.10

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Jennifer J. Puente

PROVISIONAL APPLICATION COVER SHEET
Additional Page

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Docket Number 112 PRO (1087-96)

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NOVEL ANTI-DC-SIGN ANTIBODIES

TECHNICAL FIELD

The present disclosure relates to compositions useful in modulating, i.e., increasing or reducing, the immune response in an animal.

BACKGROUND

Dendritic cells (DC) are professional antigen-presenting cells that capture antigens in the peripheral tissues and migrate via lymph or blood to the T cell area of the draining lymph nodes and spleen. They present processed antigens to naïve T cells, initiating antigen-specific primary T cell responses. DC are unique in their ability to interact with and activate resting T cells.

Naïve T cells are characterized by a high expression of ICAM-3 which is a member of the IgG supergene family and is rapidly downregulated after activation (Vazeux et al., "Cloning and characterization of a new intercellular adhesion molecule ICAM-R", Nature, 360, pp 485-488 (1992)).

C-type lectins are calcium-dependent carbohydrate binding proteins with a wide range of biological functions, many of which are related to immunity. Recently, a novel ICAM-3 binding C-type lectin, known as DC-Specific ICAM-3 grabbing non-integrin, or DC-SIGN, was found. DC-SIGN is expressed on DCs and appears to mediate adhesion between dendritic cells and ICAM-3 on naïve T cells and to be essential for DC-induced T cell proliferation (Geijtenbeek et al., "Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses", Cell, vol. 100, no. 5, pp. 575-585 (2000); Steinman, "DC-SIGN: A guide to some mysteries of dendritic cells", Cell, vol. 100, no. 5, pp. 491-494 (2000)). Binding of antibodies to DC-SIGN can result in internalization (Engering, et al., "The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells," *J Immunol 168:2118* (2002)).

WO 00/63251, the contents of which are incorporated by reference herein, discloses that immune responses can be inhibited or prevented by preventing the interaction of DC-SIGN on dendritic cells with receptors on T cells, e.g., by using antibodies specific for DC-SIGN. Alternatively, an immune response to an antigen can be potentiated by binding an antigen to DC-

SIGN on dendritic cells such that the antigen plus DC-SIGN is taken up by dendritic cells and processed and presented to T cells.

Besides its prominent role in DC-T cell clustering and initiation of T cell responses, DC-SIGN is a major receptor involved in infection of DC and subsequent transmission to T cells of viruses such as HIV-1, HIV-2, SIV-1, hepatitis C virus (HCV), Ebola virus, cytomegalovirus (CMV), and Dengue virus; bacteria such as Helicobacter pylori, Klebsiella pneumonae, and Mycobacteria tuberculosis; yeast such as Candida albicans; and parasites such as Leishmania pifanoi and Schistosoma mansoni. Due to their position in the body surface as immunosurveillance cells, it is likely that DC are the first cells infected with these viruses after mucosal exposure and therefore play an important role in the immunopathogenesis of diseases caused by these viruses. It is now generally believed that these viruses, such as HIV, convert the normal trafficking process of DC to gain entry into lymph nodes and access to $CD4^+$ T cells. For example, productive infection of DC with HIV-1 has been reported by several investigators (Granelli-Piperno et al., J Virol 72(4), 2733-7 (1998); Blauvelt et al., Nat Med 3(12), 1369-75 (1997)), and substantial evidence indicates that DC pulsed with HIV-l in vitro can induce a vigorous infection when co-cultured with T cells (Cameron et al., Science 257(5068), 383-7 (1992)). Although it is still unclear whether a similar scenario occurs in HIV infected individuals, HIV-1 transmission from DC to T cells could contribute to the $CD4^+$ T cell depletion observed in AIDS. Thus these viruses, such as HIV-1, and resting T cells exploit a similar highly expressed receptor to interact with DC.

Specific compositions useful in increasing or reducing the immune response in an animal remain desirable.

SUMMARY

The present disclosure is directed to novel anti-DC-SIGN antibodies which can be useful in modulating the immune response of an animal.

In one embodiment, the anti-DC-SIGN antibodies interfere with the interaction of DC-SIGN expressing cells and ICAM-expressing cells. More specifically, in this embodiment the anti-DC-SIGN antibodies reduce the adhesion of C-type lectin receptors on the surface of dendritic cells to ICAM receptors on the surface of T cells. By modulating this adhesion,

dendritic cell-T cell interactions can be influenced. Such interactions include cluster formation and antigen presentation, as well as primary T cell responses dependant thereon, resulting in a modulation of the immune response.

In another embodiment, the anti-DC-SIGN antibodies influence the migration of DC-SIGN expressing cells.

In another embodiment, the anti-DC-SIGN antibodies of the present disclosure can act as an agonist thereby enhancing T-cell response in an animal.

In another embodiment, the anti-DC-SIGN antibodies of the present disclosure may also be used to enhance the immune response to specific peptides, especially antigens. In such a case the anti-DC-SIGN antibodies are attached to a peptide and the combination of the two are administered to an animal. The antibodies direct the peptide to dendritic cells, which internalize the peptide and then present it on the dendritic cell surface to T cells, thereby generating an immune response to the peptide. In this case the antibodies can be useful as vaccines, including cancer vaccines.

In yet another embodiment, the anti-DC-SIGN antibodies of the present disclosure can be labeled with a toxin to DC-SIGN expressing cells. Administration of the anti-DC-SIGN antibodies labeled with toxin can then be utilized to reduce the levels of DC-SIGN expressing cells which, in some instances, can be beneficial, such as in the treatment of autoimmune disease.

In another embodiment, the anti-DC-SIGN antibodies of the present disclosure inhibit infection of DC by viruses such as HIV-1, HIV-2, SIV-1, hepatitis C virus (HCV), Ebola, cytomegalovirus (CMV), and Dengue; bacteria such as Helicobacter pylori, Klebsiella pneumonae, and Mycobacteria tuberculosis; yeast such as Candida albicans; and parasites such as Leishmania pifanoi and Schistosoma mansoni. In some embodiments the anti-DC-SIGN antibodies of the present disclosure can be utilized as vaccines to diseases caused by the above-referenced viruses. In a further embodiment, the anti-DC-SIGN antibodies of the present disclosure can be used in the treatment of HIV-infections and similar disorders of the immune system, as well as to modulate the immune response to grafts or after transplant.

In some embodiments the anti-DC-SIGN antibodies of the present disclosure can be a humanized antibody. In other embodiments, the anti-DC-SIGN antibodies of the present disclosure can be an scFv.

Further embodiments of the present disclosure relate to prophylactic techniques as well as diagnostic techniques using the compositions and/or embodying the methods as described above. Compositions comprising the anti-DC-SIGN antibodies of the present disclosure in a pharmaceutically acceptable carrier are also provided.

BRIEF DESCRIPTION OF THE FIGURES

FIGs. 1A and 1B are graphical depictions of the results of in vitro experiments in accordance with the present disclosure showing the reactivity of IgG1 clones and IgG2a clones, respectively, with human DC-SIGN.

FIG. 2 is a graphical depiction of the results of in vitro experiments in accordance with the present disclosure showing the reactivity of IgG1 clones and IgG2a clones with human L-SIGN and DC-SIGN.

FIG. 3 is a graphical depiction of the results of FACS analysis of 3 clones obtained in experiments in accordance with the present disclosure showing the reactivity of the clones with DC-SIGN on the surface of dendritic cells.

FIGs. 4a-4c provide the amino acid sequences of heavy chain clones and light chain clones obtained in experiments in accordance with the present disclosure that are reactive with DC-SIGN.

FIGs. 5A-5B are graphical depictions of the results of in vitro experiments in accordance with the present disclosure showing the reactivity of IgG1 clones and IgG2a clones, respectively, competing with AZN-D1 for binding to DC-SIGN.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present disclosure is based on the finding that antibodies directed against DC-SIGN can modulate the interaction of T cells with dendritic cells. In general, the anti-DC-SIGN antibodies of the present disclosure can bind, adhere to (preferably in a reversible manner), or serve as a ligand for DC-SIGN or natural variants or equivalents thereof.

The amino acid sequence of DC-SIGN is known and reported, for example, as shown in SEQ ID No. 1 and Figure 9 in WO OO/63251.

According to the present disclosure, the anti-DC-SIGN antibodies preferably include an antibody directed against DC-SIGN, or a part, fragment or epitope of DC-SIGN. As used herein, the term antibodies includes polyclonal, monoclonal, chimeric and single chain antibodies, as well as fragments (Fab, Fv, scFv, Fc) and Fab expression libraries. Such antibodies against DC-SIGN can be obtained as described hereinbelow or in any other manner known per se, such as those described in WO 95/32734, WO 96/23882, WO 98/02456, WO 98/41633 and/or WO 98/49306.

For instance, polyclonal antibodies can be obtained by immunizing a suitable host such as a goat, rabbit, sheep, rat, pig or mouse with DC-SIGN or an immunogenic portion, fragment or fusion thereof, optionally with the use of an immunogenic carrier (such as bovine serum albumin) and/or an adjuvant such as Freund's, saponin, ISCOM's, aluminum hydroxide or a similar mineral gel, or keyhole limpet hemocyanin or a similar surface active substance. After an immunoresponse against DC-SIGN has been raised (usually within 1-7 days), the antibodies can be isolated from blood or serum taken from the immunized animal in a manner known per se, which optionally may involve a step of screening for an antibody with desired properties (i.e. specificity) using known immunoassay techniques, for which reference is again made to WO 96/23882.

Monoclonal antibodies may be produced using continuous cell lines in culture, including hybridoma and similar techniques, again essentially as described in the above cited references. In a further aspect, the present disclosure provides a cell line such as a hybridoma that produces antibodies, preferably monoclonal antibodies, against DC-SIGN.

In one embodiment, the antibody of the present disclosure comprises a light chain. As used herein, "light chain" means the smaller polypeptide of an antibody molecule composed of one variable domain (VL) and one constant domain (CL), or fragments thereof. In another embodiment, the portion of the antibody comprises a heavy chain. As used herein, "heavy chain" means the larger polypeptide of an antibody molecule composed of one variable domain (VH) and three or four constant domains (CH1, CH2, CH3, and CH4), or fragments thereof.

In another embodiment, the antibody comprises a Fab portion of the antibody. As used herein, "Fab" means a monovalent antigen binding fragment of an immunoglobulin that consists of one light chain and part of a heavy chain. It can be obtained by brief papain digestion or by recombinant methods. In another embodiment, the portion of the antibody comprises a $F(ab')_2$ portion of the antibody. As used herein, "$F(ab')_2$ fragment" means a bivalent antigen binding fragment of an immunoglobulin that consists of both light chains and part of both heavy chains. It can be obtained by brief pepsin digestion or recombinant methods. In other embodiments, the antibody may be a Fab' fragment. Fab expression libraries may for instance be obtained by the method of Huse et al., Science 245: 1275 (1989).

Furthermore, "humanized" antibodies may be used, for instance as described WO 98/49306. As used herein, "humanized" antibodies are those antibodies wherein amino acids outside the CDR are replaced with corresponding amino acids derived from human immunoglobulin molecules. "CDR" or "complementarity determining region" means a highly variable sequence of amino acids in the variable domain of an antibody. U.S. Patent No. 5,225,539 describes one approach for the production of humanized antibodies. Recombinant DNA technology can be used to produce a humanized antibody wherein the CDRs of a variable region of one immunoglobulin are replaced with the CDRs from an immunoglobulin with a different specificity such that the humanized antibody recognizes the desired target but is not recognized in a significant way by the human subject's immune system. Specifically, site directed mutagenesis is used to graft the CDRs onto the framework.

Other approaches for humanizing antibodies are described in U.S. Patent Nos. 5,585,089 and 5,693,761 and WO 90/07861. These antibodies have one or more CDRs and additional amino acids from a donor immunoglobulin and a framework region from an accepting human immunoglobulin. Specifically, these patents describe the preparation of a humanized antibody that binds to a receptor by combining the CDRs of a mouse monoclonal antibody with human immunoglobulin framework and constant regions. Human framework regions can be chosen to maximize homology with the mouse sequence. A computer model can be used to identify amino acids in the framework region which are likely to interact with the CDRs or the specific antigen and then mouse amino acids can be used at these positions to create the humanized antibody.

In one embodiment, the antibody includes one or more CDR domains of the antibody. In another embodiment, the antibodies utilized in the present disclosure are humanized antibodies having a light chain variable region comprising at least one CDR selected from the group consisting of amino acid sequences of SEQ ID NO: __, __ and __. In yet another embodiment, the antibodies utilized in the present disclosure are humanized antibodies having a heavy chain variable region comprising at least one CDR selected from the group consisting of amino acid sequences of SEQ ID NO: __, __ and __.

It is envisaged that the novel antibodies of the present disclosure will have broad applicability (i.e., besides the pharmaceutical/therapeutic uses disclosed herein). Some of these applications, which form yet another aspect of the present disclosure, will be clear to the skilled person from the disclosure herein.

Once obtained, the antibodies described above can be administered to an animal. In one embodiment, the anti-DC-SIGN antibodies reduce the immune response in an animal, in particular a human or another mammal. By binding to DC-SIGN, the antibodies impede the interaction(s) between DC-SIGN-expressing cells and ICAM-expressing cells, e.g., the interaction between a dendritic cell and a T cell. More specifically, the antibodies to DC-SIGN reduce the adhesion between a dendritic cell and a T cell by interfering with the adhesion between DC-SIGN and an ICAM receptor on the surface of a T cell.

As used herein, "ICAM receptor(s)" means both the ICAM-2 and ICAM-3 receptor, especially the ICAM-3 receptor.

By interfering with the adhesion of T cells to dendritic cells, the use of antibodies to DC-SIGN will affect dendritic cell-T cell clustering, T cell activation and other interactions that rely on contact between dendritic cells and T cells. These other interactions include both direct cell-to-cell contact or close proximity of dendritic cells and T cells.

Such further interactions include, but are not limited to, processes involved in generating an immune response, in particular during the initial stages of such a response, such as primary sensitization/activation of T lymphocytes, i.e., presentation of antigen and/or MHC-bound peptides to T cells and co-stimulation of T cells. In addition, such interactions include processes such as chemical signaling, endocytosis and transepithelial transport. For a discussion of dendritic cell-T cell interactions in general, all of which may be influenced by the compositions

of the present disclosure, reference is made to the discussion below as well as to WO 95/32734 and WO 96/23882.

Furthermore, the antibodies of the present disclosure can be used to prevent or reduce the transfer of matter from dendritic cells to T cells, such as chemicals, signaling factors such as chemokines and/or interleukins, etc., and in particular of viral particles such as HIV. In this way, by using the antibodies of the present disclosure, not only can the initial adhesion of HIV to dendritic cells be inhibited, but also the spread of HIV infection from dendritic cells to T cells.

The antibodies of the present disclosure can not only be used to prevent HIV infection of dendritic cells, but also to reduce the spread of HIV infection to T cells after the dendritic cells have been infected, thereby slowing down the disease process. The antibodies may also be used to prevent, inhibit or at least delay T cell activation and thereby slow the onset and/or the progress of a disease such as HIV.

The antibodies of the present disclosure can therefore be used to influence the immunomodulatory ability of dendritic cells; to modulate, and in particular reduce, dendritic cell-mediated (primary) T cell responses, and/or generally to influence, and in particular inhibit, the immune system. The antibodies can be used for preventing and/or treating disorders of the immune system, as well as to prevent transplant rejection.

Some additional applications include preventing or inhibiting immune responses to specific antigens; inducing tolerance; immunotherapy; immunosuppression, i.e., to prevent transplant rejection; the treatment of auto-immune diseases such as thyroiditis, rheumatoid arthritis, systemic lupus erythematosus (SLE), multiple sclerosis and auto-immune diabetes; and the prevention or treatment of allergies.

The antibodies of the present disclosure constitute a very useful diagnostic and research tool, for use both in vitro as well as in vivo. Possible non-limiting fields of application include the study of dendritic cells and their function and interactions; the study of the immune system; the detection of dendritic cells and/or C-type lectins in cells, tissues or biological fluids such as synovial tissue and skin tissue/skin cells; as well as the study of the role dendritic cells play in biological processes or disease mechanisms, such as cancer and auto-immune diseases (including, e.g., rheumatoid arthritis).

The antibodies of the present disclosure can be used to detect the presence of (and thereby determine the expression of) DC-SIGN in or on tissues or whole cells, as well as detect the presence of DC-SIGN in other biological samples such as cell fragments or in cell preparations. The information thus obtained can then be used to determine whether the method or compositions of the present disclosure can be applied to such tissues or cells. The antibodies of the present disclosure could also be used to detect (qualitatively and/or quantitatively), isolate, purify and/or produce dendritic cells, for instance in/from biological samples, including biological fluids such as blood, plasma or lymph fluid; tissue samples or cell samples such as bone marrow, skin tissue, tumor tissues, etc; or cell cultures or cultivating media. Detection can be by suitable assays.

Assays could be used in a manner known per se for the analysis of antibodies, such as competitive inhibition assays or ELISA-type immunoassays. For instance, the antibodies could be used in combination with microscopy techniques, cell sorting techniques including flow-cytometry and fluorescence activated cell sorting (FACS), techniques based upon solid supports and/or detectable labels or markers (which can be attached to the antibodies), techniques based upon (para)magnetic beads or any other detection or assay technique known to one skilled in the art in which antibodies can be used. Such assays and kits for therein, which besides the antibodies of the present disclosure can contain additional components known for antibody-based assays, as well as manuals etc., form a further aspect of the present disclosure.

By using the antibodies of the present disclosure, dendritic cells can also be isolated and produced with higher yield and with higher specificity. In such a method, the antibodies can be used in a manner known per se for the harvesting, isolation and/or purification of cells from biological fluids using antibodies.

For a further description of the methods and known techniques in which the antibodies of the present disclosure can be used, reference is made to the general textbooks, such as Sites, et al., "Basic and clinical immunology", 8th Ed., Prentice-Hall (1994); Roitt, et al., "Immunology", 2nd. Ed., Churchill Livingstone (1994); the contents of which are incorporated by reference herein. Particular reference is made to the general uses of antibodies and techniques involved therein as mentioned in sections 2.7 to 2.17 of the general reference work by Janeway-Travers: "Immunobiology, the immune system in health and disease", Third Edition.

The present disclosure further relates to a method for the prevention or treatment of HIV infections, comprising administering to a HIV infected patient or a person at risk of becoming HIV infected, a compound that can binds or bind to DC-SIGN on the surface of a dendritic cell, in such an amount that the adhesion of HIV to the dendritic cells is inhibited.

Also, the present disclosure further relates to a method for the treatment of HIV infections, comprising administering to a HIV infected patient a compound that binds or can bind to DC-SIGN in such an amount that the transfer of HIV from infected dendritic cells to non-infected T cells is inhibited.

In another aspect, antibodies of the present disclosure are used to modulate, and in particular generate, increase and/or promote, an immune response in an animal, such as a human or another mammal, against a specific peptide, i.e., an antigen or combination of antigens, by presenting said antigen(s) or one or more antigenic parts thereof to dendritic cells in a form that can bind to DC-SIGN. The antigen(s) presented in this manner are internalized, i.e., they enter the dendritic cell, which then presents the antigen on its surface to the T cells, thereby causing an immune response against the antigen(s).

The phrase "a form that can bind to DC-SIGN" with respect to presentation to dendritic cells is generally meant that the antigen or antigenic fragment is attached to the anti-DC-SIGN antibodies described above. Said attachment can be by covalent binding, ligand-ligand interaction, complexing, ligation, fusion of proteins (e.g. through expression of said fusions), or by any other type of physical or chemical interaction or bond that enables the antigen to be presented to a dendritic cell in conjunction with the anti-DC-SIGN antibodies.

The antigen can be any antigen against which an immune response is to be obtained, or any part or fragment thereof. Preferably, any such part or fragment is such that it is per se capable of eliciting an immune response, such as an epitope. However, this is not required: because the fragments are directed to the dendritic cells with increased specificity or affinity by virtue of their attachment to the anti-DC-SIGN antibodies of the present disclosure, fragments that would normally be incapable of eliciting an immune response may provide an immune response when used in conjunction with anti-DC-SIGN antibodies described herein. Also, in general, using an antigen in combination with the anti-DC-SIGN antibodies may increase the potency of the antigen, i.e., provide a higher or stronger immune response per unit of antigen

administered. In this way, antigens could be administered at a lower dosage and still provide sufficient immune response.

Examples of suitable antigens are cancer antigens including gp 100, g250, p53, MAGE, BAGE, GAGE, MART 1, Tyrosinase related protein 11 and Tyrosinase related protein; all of which can be used to generate an immune response against the tumor cells that contain or express said antigen. Other types of antigens that can be used in the present disclosure include any non-self peptides and essentially all antigens used in vaccines against infectious diseases, such as influenza, mumps, measles, rubella, diphtheria, tetanus, diseases due to infection with micro-organisms such as Haemophilus influenzae (e.g. type b), Neisseria, Bordetella pertussis, Polyomyletus, Influenza virus and Pneumococcus, and generally any other infection or disease against which a vaccine can be developed or can be envisaged, including also parasite, protozoan and/or viral infections such as HIV and herpes. To provide serums or vaccines, the compounds of the present disclosure may further be combined with other antigens known per se.

This aspect of the present disclosure therefore relates to compositions including a combination of: 1) an anti-DC-SIGN antibody and 2) an antigen or a fragment or part thereof attached thereto. The combination of the two may be utilized in a composition for modulating, in particular generating, increasing and/or promoting, an immune response in an animal, particularly a human or another mammal, against said antigen. This technique could be especially advantageous in cancer vaccines.

The above combinations can be in the form of a complex, a chemical substance or entity, or a fused protein or protein structure, and can be formulated and administered as a composition in a manner known to one skilled in the art. Thus, once obtained, the above antibodies to DC-SIGN in combination with a peptide may be administered to a host animal to boost T cell response and thus the immune response of the host animal.

In a further aspect the present disclosure relates to a method for modulating the immune response in an animal, in particular a human or another mammal, comprising administering to said animal antibodies to DC-SIGN in combination with a peptide, preferably in the form of a composition as described above, in an amount sufficient to alter or modify an immune response. Preferably this method generates an immune response to the peptide.

Compositions for administration in accordance with the present disclosure, regardless of their intended effect, may contain one or more of the abovementioned anti-DC-SIGN antibodies, or such antibodies in combination with other compounds. In some embodiments, an antibody can be formulated with mannose, fucose or other carbohydrates, lectins and/or antibiotics such as pridamicin A, whereby a synergistic effect may be obtained.

The compositions of the present disclosure may also contain or be used in combination with known co-inhibitory compounds, such as anti-LF3A; as well as other active principles known per se, depending upon the condition to be treated. For instance, the compositions of the present disclosure may be formulated or used in combination with immunosuppressants (i.e. for preventing transplant rejection), immunomodulants, antibiotics, auto-antigens or allergens (for instance as described in WO 95/3234 or WO 96/23882), Tumor Necrosis Factor (TNF), and anti-viral agents such as anti-HIV agents and CD4 inhibitors including CD4 directed antibodies such as Leu-3A, whereby a synergistic effect can also be obtained.

The compositions of the present disclosure can be formulated using known carriers and/or adjuvants to provide a pharmaceutical form known per se, such as a tablet, capsule, powder, freeze-dried preparation, solution for injection, etc., preferably in a unit dosage form. Such pharmaceutical formulations of antibodies, their use and administration (single or multi-dosage form), as well as carriers, excipients, adjuvants and/or formulants for use therein, are generally known in the art and are for instance described in WO 93/01820, WO 95/32734, WO 96/23882, WO 98/02456, W098/41633 and/or WO 98/49306, the contents of each of which are incorporated by reference herein. Furthermore, the formulation can be in the form of a liposome, as described in WO 93/01820.

The compositions of the present disclosure may further be packaged, for instance in vials, bottles, sachets, blisters, etc.; optionally with relevant patient information leaflets and/or instructions for use.

In all the above methods, the compounds/compositions used will be administered in a therapeutically effective amount, for which term reference is generally made to WO 93/01820, WO 95/32734 and/or WO 96/23882, the contents of which are incorporated by reference herein. The administration can be a single dose, but is preferably part of a multi-dose administration regimen carried out over one or more days, weeks or months.

Kits according to the present disclosure include frozen or lyophilized antibodies to be reconstituted, respectively, by thawing (optionally followed by further dilution) or by suspension in a (preferably buffered) liquid vehicle. The kits may also include buffer and/or excipient solutions (in liquid or frozen form), or buffer and/or excipient powder preparations to be reconstituted with water, for the purpose of mixing with the antibodies to produce a formulation suitable for administration as a therapeutic. Thus, preferably the kits containing the antibodies are frozen, lyophilized, pre-diluted, or pre-mixed at such a concentration that the addition of a predetermined amount of heat, water, or solution provided in the kit will result in a formulation of sufficient concentration and pH as to be effective for in vivo or in vitro use. Preferably, such a kit will also comprise instructions for reconstituting and using the antibody composition as a therapeutic. The kit may also comprise two or more component parts for the reconstituted active composition. For example, a first component can include the antibodies and the second component can include a bifunctional chelate or a therapeutic agent such as a radionuclide, which when mixed with the antibodies forms a conjugated system therewith. The above-noted buffers, excipients, and other component parts can be sold separately or together with the kit.

Furthermore, although the present disclosure is described herein with respect to antibodies to DC-SIGN, it is not excluded that other, generally similar C-type lectins, including natural variants of DC-SIGN, may also be present on dendritic cells and/or may be involved in dendritic cell - T cell interaction. Such variants will usually have a high degree of amino acid homology (more than 80% to more than 90%) with, and/or be functionally equivalent to DC-SIGN. Thus, in some cases it is anticipated that the anti-DC-SIGN antibodies of the present disclosure may also bind to such variants, thereby altering DC-T cell interaction as described above with respect to DC-SIGN.

The following non-limiting examples are provided to illustrate the present disclosure.

EXAMPLE 1

Library construction

Mice were immunized with immature dendritic cells obtained by maturing primary blood lymphocytes with 500 U/ml IL-4 and 800 U/ml GM-CSF for 6 days. After 3 immunizations, the spleen was harvested and homogenized in TRI reagent (Molecular Research Center). Total RNA was isolated according to the manufacturer's instruction. Messenger RNA was purified using

Oligotex (QIAGEN). First strand cDNA was synthesized using SUPERSCRIPT First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies) according to the manufacturer's protocol. First strand cDNA was mixed with oligonucleotides: mCG1Xcm I for IgG1, mCG2asBsaJ I for IgG2a, and mCKHpa I for kappa light chain in separate tubes and digested with Xcm I (for IgG1), BsaJ I (for IgG2a) and Hpa I (for kappa light chain). The sequences of these oligonucleotides are set forth below:

mCG1Xcm I	5'CTAACTCCATGGTGACCCTGGGATG3'	SEQ ID NO. __
mCG2aBsaJ I	5'CAACTGGCTCCTCGGTGACTCTAG3'	SEQ ID NO. __
mCKHpa I	5'CAGTGAGCAGTTAACATCTGGAGG3'	SEQ ID NO. __

Quality and full digestion of the first strand cDNA was checked with PCR using primers that annealed both externally and internally to the digestion site.

Second strand cDNA synthesis was performed using primers that possessed a portion that hybridized to the framework 1 region of heavy chain and light chain genes, a restriction enzyme site, and a non-hybridizing predetermined sequence. Second strand synthesis was repeated for 20 cycles consisting of 94°C for 5 seconds, 56°C for 10 seconds, and 68°C for 2 minutes.

At the end of the cycle, oligonucleotides (TMX24mCG1noer, TMX24mCG2anoer, and TMX24mCKnoer) for extension reactions were added on ice and the synthesized cDNAs were further extended along these oligonucleotides by incubating them at 94°C for 1 minute and then at 68°C for 2 minutes. The oligonucleotides used for the extension reaction had a portion that hybridized to the constant region of the antibody gene, a restriction enzyme site, and a non-hybridizing predetermined sequence. A nucleotide at the very 3' end of the oligonucleotide was non-hybridizing and three 3' end nucleotides were modified with phosphorthioate and 2' OMe linked propyl group on the 3' end which prevented extension along the synthesized second strand cDNA and made it protective against exo- and endonuclease activity. The sequences of these oligonucleotides are set forth below:

TMX24mCG1noer

5'GACGTGGCCGTTGGAAGAGGAGTGCCTAGGGTTACCATGGAGTTAGTTTGGGCAG
CAGA2'OMe[U(ps)C(ps)A(ps)](propyl) 3' SEQ ID NO. __

TMX24mCG2anoer

5'GACGTGGCCGTTGGAAGAGGAGTGCCTAGGGTCATCGAGGAGCCAGTTGTATCTC
CACA2'OMe[C(ps)A(ps)U(ps)](propyl) 3' SEQ ID NO. __

TMX24mCKnoer

5'GACGACCGGCTACCAAGAGGAGTGTCCGGATGTTAACTGCTCACTGGATGGTGGG
AAGATGG2'OMe[A(ps)U(ps)U(ps)](propyl) 3' SEQ ID NO. __

After the extension reaction was completed, the reaction was then cooled to 4°C and cleaned by a PCR purification kit (QIAGEN).

Single primer amplification was then performed using primers (TMX24mH and TMX24mK) that had the same predetermined sequences used for the primers for the second strand cDNA synthesis and oligonucleotides for the extension reaction. The sequences of the primers for single amplification were selected so that they have no significant homology to the known mouse gene and were as follows:

TMX24mH 5'GACGTGGCCGTTGGAAGAGGAGTG3' SEQ ID NO. __

TMX24mK 5'GACGACCGGCTACCAAGAGGAGTG3' SEQ ID NO. __

Amplified products were purified with PCR purification kit and digested with Xho I/Bln I (IgG1 and IgG2a) and Xba I/BspE I (kappa light chain) and cloned into a PAX313m/hG vector.

EXAMPLE 2

Panning

The IgG 1 and IgG2a libraries were panned on recombinant human DC-SIGN-Fc. Phage (10^{12}) were incubated in 2 wells of a 96 well plate coated with 1 μg/ml DC-SIGN captured by anti-human Fc antibody. After 2 hours of incubation at 37°C, the wells were washed 3 times with phosphate buffered saline (PBS) for the 1st round of panning, 5 times for the 2nd round of panning and 10 times for the 3rd round of panning, with 5 minute intervals between washes. Bound phage was eluted with 0.1 M HCL containing 1 mg/ml bovine serum albumin (BSA) at pH 2.2. Erythrocyte rosette (ER) cells were infected with the eluate and cultured in the presence of carbicillin and tetracycline for 2 hours before the addition of isopropylthio-β-D-galactoside (IPTG) and helper phage. Two hours later, kanamycin was added and the cells were grown overnight. The next day, cultures were spun down and phage was precipitated from the supernatant using polyethylene glycol/sodium chloride (PEG/NaCl).

EXAMPLE 3

Solid phase ELISA

ELISA plates were coated overnight at room temperature with 2 μg/ml of anti-human Fc in PBS. Following 3 rounds of washing with PBS containing 0.05% Tween, the plates were blocked with PBS containing 1%BSA. After 1 hour at 37°C, plates were washed 3 times and incubated for 2 hours with 500 ng/ml recombinant human DC-SIGN-Fc protein. Culture supernatants from clones grown overnight in SB containing 1μg/ml carbicillin were added for 2 hours. After 3 washes, bound antibody was detected with alkaline phosphatase conjugated anti-mouse Fab'2 antibody followed by the addition of SigmaS substrate. Color development was detected at OD 405 using a plate reader (Molecular Devices).

For the IgG2a library, 47/291 clones showed a 4-13-fold increase in signal to DC-SIGN over BSA. The IgG 1 library yielded 22/240 clones from screened pan 3 exhibiting a strong signal on DC-SIGN, which was up 23-fold stronger over the BSA signal. A representative example for a 96 well plate for each library is shown in Figure 1.

Clones showing a signal on DC-SIGN were examined for their reactivity with DC-SIGNR (L-SIGN), a highly related protein. The ELISA was performed similar to the DC-SIGN

ELISA except that L-SIGN instead of DC-SIGN was coated on the plate. As shown in Figure 2, clones from the IgG1 library were highly specific for DC-SIGN. Clones from the IgG2a library generally showed a lower signal and some clones did cross-react with L-SIGN.

EXAMPLE 4

FACS analysis

To verify that the clones reacting with recombinant DC-SIGN also recognized DC-SIGN on the cell surface, immature dendritic cells were incubated with culture supernatant of the clones of interest and diluted 1:1 with FACS buffer (PBS containing 1% BSA and 0.1% NaN_3) for 20 minutes on ice. Cells were washed twice with FACS buffer and cell surface bound antibody was detected with a PE-conjugated anti-mouse IgG antibody. Samples were analyzed using a FACS Calibur (Becton Dickinson). The results for some representative samples are set forth in Figure 3. As seen in Figure 3, all samples recognized the protein on immature dendritic cells.

EXAMPLE 5

Sequences

DNA from 16 clones from each library giving a positive signal in solid phase ELISA was isolated and submitted for sequencing to Retrogen, Inc. (San Diego, CA). Sequences obtained are set forth in Figures 4a-c. Only unique sequences are shown. As seen in Figure 4a, light chain CDR3 regions that will bind to human DC-SIGN have one of the following sequences: QHFWNTPWT (SEQ ID NO __); and QQGHTLPYT (SEQ ID NO __). As seen in Figure 4b, heavy chain CDR3 regions that will bind to human DC-SIGN have one of the following sequences: SNDGYYS (SEQ ID NO __); RYYLGVD (SEQ ID NO __); DDSGRFP (SEQ ID NO __).

As seen in Fig 4c, the heavy chain CDR3 regions of the antibodies that bind to human DC-SIGN can also have one of the following amino acid sequences: YGYAVDY (SEQ ID NO __); YYGIYVDY (SEQ ID NO __); FLVY (SEQ ID NO __); NFGILGY (SEQ ID NO __); YPNALDY (SEQ ID NO __); or GLKSFYAMDH(SEQ ID NO __). As also seen in Fig 4c, the light chain CDR3 regions of the antibodies that bind to human DC-SIGN can also have one of the following amino acid sequences: QQGKTLPWT (SEQ ID NO __); QQGNTLPPT (SEQ ID

NO __); QQHYITPLT (SEQ ID NO __); QQYGNLPYT (SEQ ID NO __); QQYYSTPRT (SEQ ID NO __); GQSYNYPPT (SEQ ID NO __); or WQDTHFPHV(SEQ ID NO __).

EXAMPLE 6

Competition ELISA

To determine whether clones reactive with DC-SIGN recognized an epitope different from AZN-D1, a known anti-DC-SIGN antibody described in WO 00/63251, a competition ELISA was performed. Plates were coated with recombinant human DC-SIGN as described above. Culture supernatants were added at various concentrations in the presence of 1 μg/ml AZN-D1. Binding of AZN-D1 was detected using an alkaline-phosphatase conjugated anti-mouse Fc antibody followed by SigmaS substrate. Color development was detected at OD 405 using a plate reader (Molecular Devices). Loss of signal indicated that the Fab and AZN-D1 competed for the same epitope. As shown in Figure 5, for the IgG1 library clones there was only weak competition with clones 1G4, 2H7 and 2B8 (2H7 and 2B8 turned out to have identical sequence). For the IgG2a library clones, clones 2,4,5,6,7,8,9,11,12,13,14 and 15 did not compete indicating that they recognized epitopes very distinct from AZN-D1. For those clones showing some competition, the epitopes recognized, while possibly still somewhat different, were close enough to AZN-D1's epitope to be blocked by the antibody.

It will be understood that various modifications may be made to the embodiments disclosed herein. For example, as those skilled in the art will appreciate, the specific sequences described herein can be altered slightly without necessarily adversely affecting the functionality of the antibody or antibody fragment. For instance, substitutions of single or multiple amino acids in the antibody sequence can frequently be made without destroying the functionality of the antibody or fragment. Thus, it should be understood that antibodies having a degree of homology greater than 70% to the specific antibodies described herein are within the scope of this disclosure. In particularly useful embodiments, antibodies having a homology greater than about 80% to the specific antibodies described herein are contemplated. In other useful embodiments, antibodies having a homology greater than about 90% to the specific antibodies described herein are contemplated. Therefore, the above description should not be construed as limiting, but merely as exemplifications of preferred embodiments. Those skilled in the art will envision other modifications within the scope and spirit of this disclosure.

ABSTRACT

Antibodies to DC-SIGN are disclosed which are capable of modulating the interaction of dendritic cells and T cells. In some embodiments, the antibodies inhibit the interaction of dendritic cells and T cells. In other embodiments, the antibodies are combined with peptides which are internalized in dendritic cells and presented to T cells, thereby generating an immune response to the peptide.

Figure 1A

IgG1 library

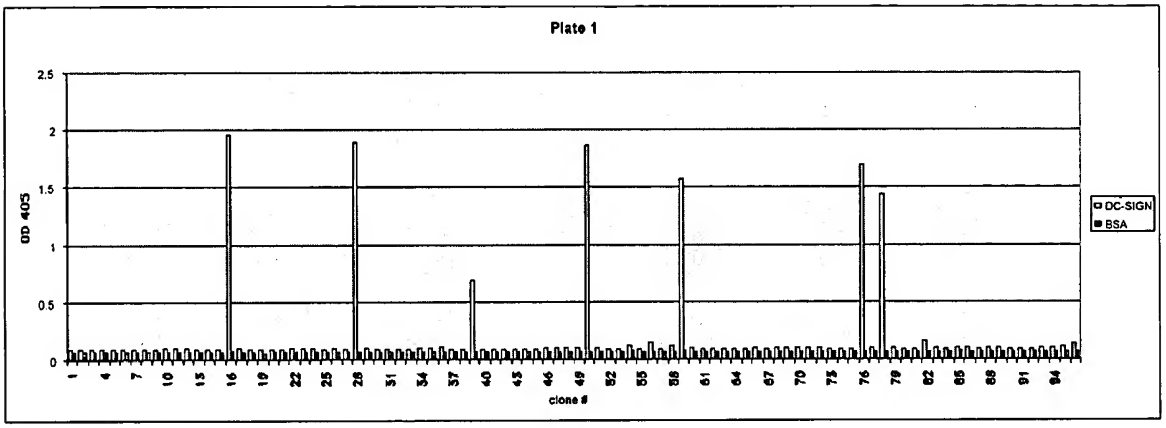


Figure 1B

IgG2a library

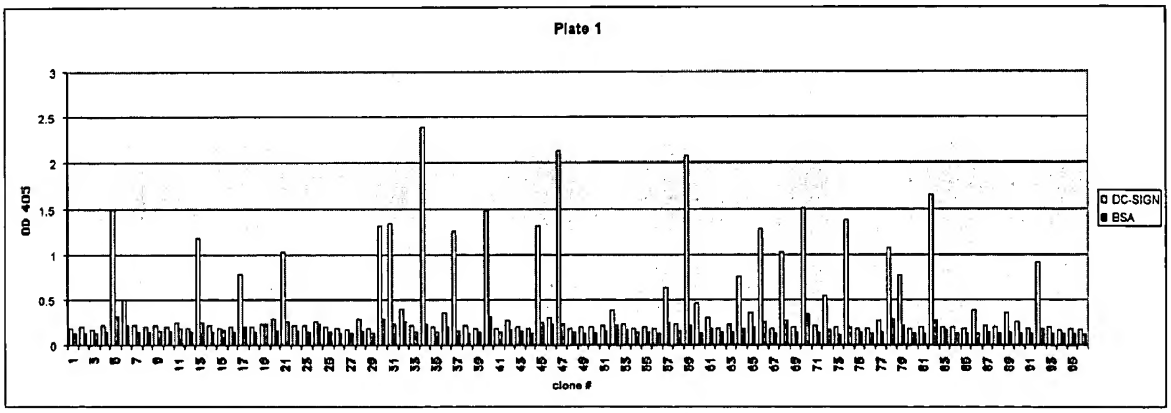


Figure 2

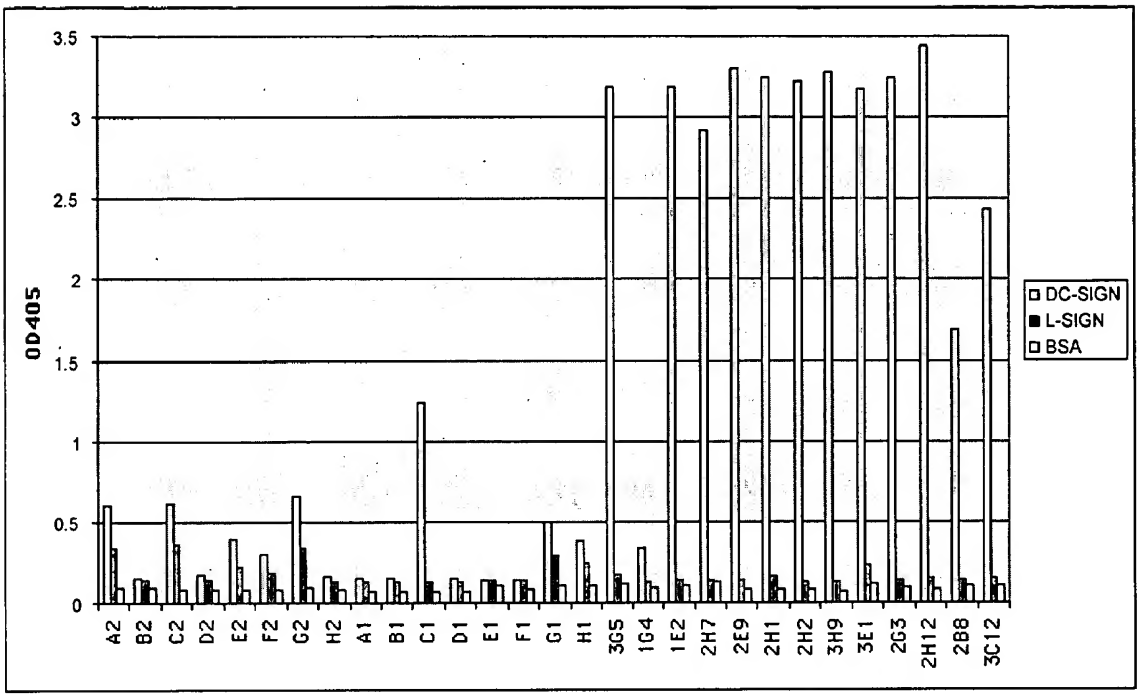
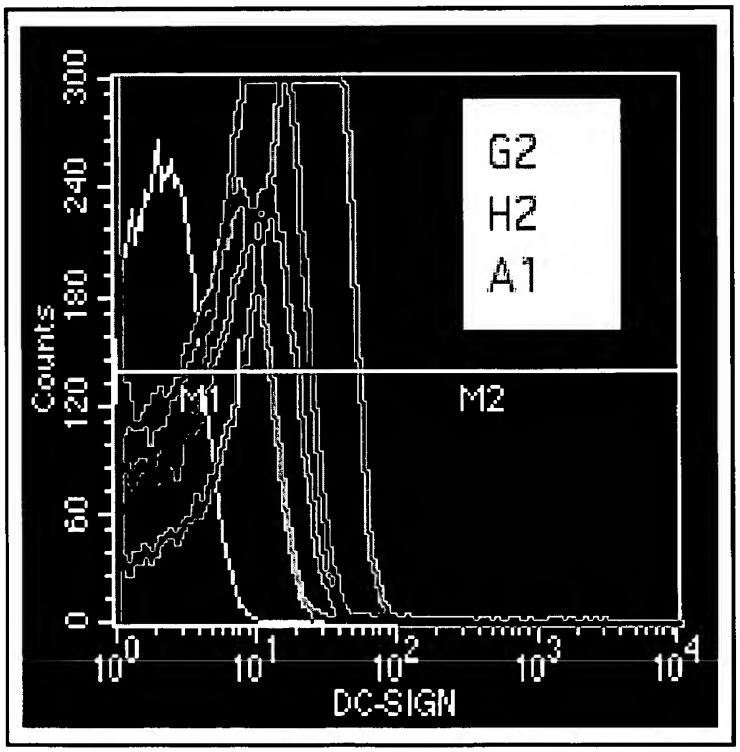


Figure 3



A1= clone 1
H2=clone 15
G2=clone 16

Figure 4a

Light chain sequences

```
     D I Q M T Q T T S S L S A S L G D R V T I T C R A S Q D I N N Y L N W Y Q Q K P  Majority
     ----------------+-----------------+-----------------+-----------------+-
                     10                20                30                40
     ----------------+-----------------+-----------------+-----------------+-
  1  D I Q M T Q S P A S L S A S V G E T I T I T C R A S E N I H N Y L A W Y Q Q N Q  1G4LC
  1  D I Q M T Q T T S S L S A S L G D R V T I T C R T S Q D I D N Y L N W Y Q Q K P  2B8LC
  1  D I P M T Q T T S S Q S A S L G D R V T I T C R A S Q D I N N Y L N W Y Q Q K P  2H1LC
  1  D I Q M T Q T T S S L S A S L G D R V T I S C R A S H D I N D Y L N W Y Q Q K P  2H2LC
  1  D I Q M T Q T T S S L S A S L G D R V T I T C R A S Q D I S N Y L N W Y Q Q K P  3C12LC
  1  D I Q M T Q T T S S Q S A S L G D R V T I T C R A S Q D I N N Y L N W Y Q Q K P  3E1LC
  1  D I Q M T Q T T S S L F A S L G D R V T I S C R A S Q D I R N N L N W Y Q Q K P  3E7LC
  1  D I Q M T Q T T S S L S A S L G D R V T I S C R A S H D I N D Y L N W Y Q Q K P  3H9LC

     D G T V K L L I Y Y T S R L H S G V P S R F S G S G S G T D Y S L T I S N L A Q  Majority
     ----------------+-----------------+-----------------+-----------------+-
                     50                60                70                80
     ----------------+-----------------+-----------------+-----------------+-
 41  G K S P Q L L V Y N A K T L A V G V P S R F S G S G S G T Q F S L K I V S L Q P  1G4LC
 41  D G T V K L L I Y Y T S R L H S G V P S R F S G S G S G T D Y S L T I N N L A Q  2B8LC
 41  D G T V K L L I Y Y T S R L H S G V P S R F S G S G S G T D Y S L T I S N L E Q  2H1LC
 41  D G T V K L L I Y Y T S S L Q S G V P S R F R G Y G S G T D Y S L T I S N L A Q  2H2LC
 41  D G T V K L L I Y Y T S R L H S G V P S R F S G S G S G T D Y S L T I S N L A Q  3C12LC
 41  D G T V K L L I Y Y T S R L H S G V P S R F S G S G S G T D Y S L T I S N L E Q  3E1LC
 41  D G T V K L L I Y Y T S S L P S G V P S R F S G S R S G T D Y S L T I S N L E Q  3E7LC
 41  D G T V K L L I Y Y T S S L Q S G V P S R F R G Y G S G T D Y S L T I S N L A Q  3H9LC

     E D I A T Y F C Q Q G D T L P W T F G G G T K L E I K R A    (SEQ ID NO ___)     Majority
     ----------------+-----------------+-----------------
                     90                100
     ----------------+-----------------+-----------------
 81  E D F G N Y Y C Q H F W N T P W T F G R G T K L E I K R A  (SEQ ID NO ___)       1G4LC
 81  E D I A T Y F C Q Q G D T L P F T F G S G T T L E I K R A  (SEQ ID NO ___)       2B8LC
 81  E D L V T Y F C Q Q G K T L P W T F G G G T K L E I K R A  (SEQ ID NO ___)       2H1LC
 81  E D F A T Y F C Q Q G H T L P Y T F G G G T K L E I K R A  (SEQ ID NO ___)       2H2LC
 81  E D I A T Y F C Q Q G D K L P F T F G S G T T L E I K R A  (SEQ ID NO ___)       3C12LC
 81  E D L A T Y F C Q Q G K T L P W T F G G G T K L E I K R A  (SEQ ID NO ___)       3E1LC
 81  E D I A T Y F C Q Q G D T L P P T F G G G T K L E I K R A  (SEQ ID NO ___)       3E7LC
 81  E D F A T Y F C Q Q G H T L P Y T F G G G T K L E I K R A  (SEQ ID NO ___)       3H9LC
```

Figure 4 b

Heavy chain sequences

```
E V Q L Q Q S G P E L V K P G A S V K I S C K A S G Y S F T - G Y Y M H W V K Q  Majority
-----------------+-----------------+-----------------+-----------------+-
                 10                20                30                40

E V Q L Q Q S G P G L V K P S Q S L S L A C S V T G Y S I T S G Y Y W N W I R Q  1G4HC
E V Q L Q Q S G P E L V K P G A S V K I S C K A S G Y S F T - G Y Y M H W V K Q  1H2HC
E V Q L Q Q S G P E L V K P G A S V K I S C K A S G Y S F T - G Y Y M H W V K Q  2B8HC
E V Q L Q Q S G P E L V K P G T S V K I S C K A S G Y S F T - G Y Y I H W V R Q  2H1HC
E V Q L Q Q S G P E L V K P G T S V K I S C K A S G Y S F T - G Y Y I H W V R Q  2H2HC
E V Q L Q Q S G P E L V K P G A S V K I S C K A S G Y S F T - G Y Y M H W V K Q  3C12HC
E V Q L Q Q S G P E L V K P G A S V K I S C K A S G Y S F T - G Y Y M H W V K Q  3E1HC
E V Q L Q Q S G A E L V R P G A L V K L S C K A S G F N I K - D Y Y I H W V K Q  3E7HC

S H V K S L E W I G R I N P Y N G A T S Y N Q N F K D K A S L T V D K S S T T V  Majority
-----------------+-----------------+-----------------+-----------------+-
                 50                60                70                80

S P G N K L E W M G Y I S Y D - G N S D Y N P S F K N R I S I T R D T S K N Q F  1G4HC
S H V K S L E W I G R I N P Y N G A T S Y N Q N F K D K A S L T V D K S S T T V  1H2HC
S H V K S L E W I G R I N P Y N G A T Y Y N H N F K D K A T L T V H K S S T T V  2B8HC
R H V K S L E W I G R I N P Y S G A T S Y N Q S F K D K A S L T V D K S S T T A  2H1HC
R H V K S L E W I G R I N P Y S G A T S Y N Q S F K D K A S L T V D K S S T T A  2H2HC
S H V K S L E W I G R I N P Y N G A T S Y N Q N F K D K A S L T V D K S S T T V  3C12HC
S H V K S L E W I G R I N P Y N G A P S Y N Q N F K D K A S L T V D E S S T T V  3E1HC
R P E Q G L E W I G W I D P E N G N T I Y D P K F Q G K A S I T A D T S S N T A  3E7HC

Y M E V H S L T S E D S A V Y Y C V R S N D G Y Y S Y P M D Y W G Q G T S V T V  Majority
-----------------+-----------------+-----------------+-----------------+-
                 90                100               110               120

F L R L N S L T T E D T A T Y Y C V R D D S G R - - F P Q W G Q G T L V T V S A  1G4HC (SEQ ID NO
__)
Y M E V H S L T S E D S A V Y Y C V R S N D G Y Y S Y P M D Y W G Q G S S V A V  1H2HC
Y M E V H S L T S E D S A V Y Y C V R S N D G Y Y S Y P M D Y W G Q G T S V T V  2B8HC
Y M E V H S L T S E D S A V Y Y C V R S N D G Y Y S Y P M D Y W G Q G T S V T V  2H1HC
Y M E V H S L T S E D S A V Y Y C V R S N D G Y Y S Y P M D Y W G Q G T S V T V  2H2HC
Y M E V H S L T S E D S A V Y Y C V R S N D G Y Y S Y P M D Y W G Q G T S V T V  3C12HC
Y M E V H S L T S E D S A V Y Y C V R S N D G Y Y S Y P M D Y W G Q G T S V T V  3E1HC
Y L Q L S S L T S E D T A V Y Y C A R Y Y L G - - - - - V D Y W G Q G T S V T V  3E7HC

S S   (SEQ ID NO ___)                                                        Majority
----

----

S S    (SEQ ID NO ___) 1H2HC
S S    (SEQ ID NO ___) 2B8HC
S S    (SEQ ID NO ___) 2H1HC
S S    (SEQ ID NO ___) 2H2HC
S S    (SEQ ID NO ___) 3C12HC
S S    (SEQ ID NO ___) 3E1HC
S S    (SEQ ID NO ___) 3E7HC
```

Figure 4c

IgG2a library
Heavy Chains

	CDR1		CDR2		CDR3		
EVQLQQSGPDLVKPGASVRISCKTSGFTFA	*NYYIH*	WVKQRPGQGLEWIG	*WIFPGNFKTEYNEKFKG*	KATLTADKSSSTAYMQLSSLTSEDSAVYFCARG	*YGYAVDY*	WGQGTSVTVSSAKTT	1 (SEQ ID NO ___)
EVQLQQSGAELVKPGASVKLSCTASGFNIK	*DTYMH*	WVKQRPEQGLEWIG	*RIDPANGNTKYDPKFQG*	KATITADTSSNTAYLQLSSLTSEDTAVYYCARD	*YYGIYVDY*	WGQGTTLTVSSAK	2 (SEQ ID NO ___)
EVQLQQSGAELARPGASVKLSCKASGYTFR	*SYWMQ*	WVKQRPGQGLEWIG	*AIYPGDGDTRYTQKFKG*	KATLTADKSSSTAYMQLSSLASEDSAVYFCARG	*GLKSFYAMDH*	WGQGTSVTVSSAK	3 (SEQ ID NO ___)
EVQLQQSGPDLVKPGASVRISCKTSGFTFA	*SYYIH*	WVKQRPGQGLEWIG	*WIFPGNFKTEYNEKFKG*	KATLTADRSSSTAYMQLSSLTSEDSAVYFCARG	*YGYAVDY*	WGQGTSVTVSSAKT	4 (SEQ ID NO ___)
EVQLQQSGPDLVKPGASVRISCKASGFTFA	*SYYIH*	WVKQRPGQGLEWIG	*WIFPGNNNTKSNEKFKG*	KATLTADKSSSTAYMQLSSLTSEDSAVYFCARG	*YGYAVDY*	WGQGTSVTVS	5 (SEQ ID NO ___)
EVQLQQSGPDLVKPGASVRISCKASGFTFA	*SYYIH*	WVKQRPGQGLEWIG	*WIFPGNNNTKSNEKFKG*	KATLTADKSSSTAYMQLSSLTSEDSAVYFCARG	*YGYAVDY*	WGQGTSVTVSSAKT	7 (SEQ ID NO ___)
EVQLQQSGAELVKPGASVKLSCTASDFNIK	*DTYIQ*	WVKQRPEQGLEWVG	*RIDPANGEIKYDPKFQG*	KATITADTSSNTAYLQLSSLTSEDTAVYYCTAY	*FLVY*	WGQGTLVTVSAAKTT	8 (SEQ ID NO ___)
EVQLKESGPGLVAPSQSLSITCTVSGFSLS	*RYTVH*	WIRQPPGKGLEWLG	*MIWGGGTTDYNSALKS*	RLSISKDNSESQVFLKMDSLQTDDTAKYYCARI	*NFGILGY*	WGQGTLVTVSAAKTT	11(SEQ ID NO ___)
EVQLQQSGTELVRPGALVKLSCKASGFNIK	*DYFMH*	WVKQRPEQGLEWIG	*WIDPKNGNTIYDPKFQV*	KASITADTSSNTAYLQLTSLTSEDTAVYYCTTT	*YPNALDY*	WGQGTSVTVSSAKTT	15(SEQ ID NO ___)

Light chains

DIAMTQSHKFMSTPVGDRVSITC	*KASQDVSTA*	VA	WYQQKPGQSPKLLIY	*SASYRYT*	GVPDRFTGSGSGTDFTFTISSVQAEDLAVYYC	*QQHYITPLT*	FGAGTKLELKR 1(SEQ ID NO ___)
DVVMTQTPLTLSVTIGQPASISC	*KSSQSLLDSDGKTYLN*	WLLWRPGQSPKRLIY	*LVSKLDS*	GVPDRFTGSGSGTDFTLKISRVEAEDLGVYFC	*WQDTHFPHV*	HVRCWDQAGTT	2(SEQ ID NO ___)
SIVLTQSPKSMSMSVGERVTLSC	*KASENVGTY*	*VS*	WYQQKPDQSPKLLIY	*GSSNRST*	GVPDRFTGSGSATDFTLTISSVQAEDLADYHC	*GQSYNYPPT*	FGGGTKLEIKR 3(SEQ ID NO ___)
DIVLTQSHKFMSTSVGDRVSITC	*KASQDVSTA*	*VA*	WYQQKPGQSPKLLIY	*WASTRHT*	GVPDRFTGSGSGTDYTLTISSVQAEDLALYYC	*QQYYSTPRT*	FGGGTKLEIKR 4(SEQ ID NO ___)
RYQMTQTTSSLSASLGDRVTISC	*SASQDITNYLN*		WYQQKPDGTVKLLLY	*YTSTLHS*	GVPSRFSGSGSGTDYSLTISNLEPEDVATYYC	*QQYGNLPYT*	FGGGTKLEIKR 5(SEQ ID NO ___)
DIQCTQTTSSLSASLGDRVTISC	*RASQDISNYLN*		WYQQKPDGTVKLLIY	*YTSRLHS*	GVPSRFSGSGSGTDYSLTISNLEQADIATYFC	*QQGKTLPWT*	FGGGTKLEIKR 7(SEQ ID NO ___)
RHQMTQSHKFMSTSVGDRVSITC	*KASQDVSTAVA*		WYQQNPGQSLKLLIY	*WASTRHT*	GVPDRFTGSGSGTDYTLTINSVQAEDLTLYYC	*QQHYITPLT*	FGAGTKLELKR 8(SEQ ID NO ___)
RYPDAQTTSSLSASLGDRVTISC	*RASQDISNYLN*		WYQQKPDGTVKLLIY	*YTSRLHS*	GVPSRFSGSGSGTDYSLTISNLEQEDIATYFC	*QQGNTLPPT*	FGGGTKLEIKR 11(SEQ ID NO ___)
DIVQTQSPASLAVSLGQRATISC	*KASQSVDYDGDSYMN*		WYQQKPGQPPKLLIY	*AASNLHS*	GVPSRFSGSGSGTDYSLTISNLEQADIATYFC	*QQGKTLPWT*	FGGGTKLEIKR 15(SEQ ID NO ___)

Figure 5A

IgG1 library

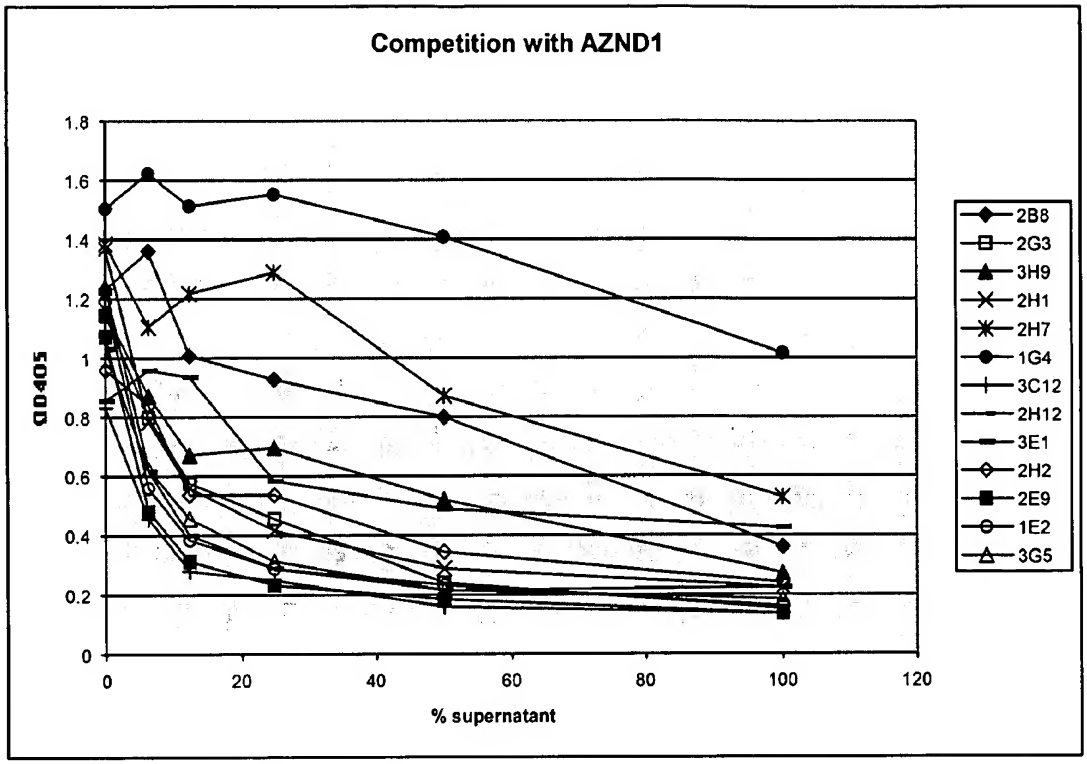


Figure 5B

IgG2a library